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BRIEF REPORT

Brief Report: Plasma Leptin Levels are Elevated in Autism: Association with Early Onset Phenotype?

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Abstract There is evidence of both immune dysregulation and autoimmune phenomena in children with autism spectrum disorders (ASD). We examined the hormone/cytokine leptin in 70 children diagnosed with autism (including 37 with regression) compared with 99 age-matched controls including 50 typically developing (TD) controls, 26 siblings without autism, and 23 children with developmental disabilities (DD). Children with autism had significantly higher plasma leptin levels compared with TD controls ($p < .006$). When further sub-classified into regression or early onset autism, children with early onset autism had significantly higher plasma leptin levels compared with children with regressive autism ($p < .042$), TD controls ($p < .0015$), and DD controls ($p < .004$). We demonstrated an increase in leptin levels in autism, a finding driven by the early onset group.

Keywords Inflammation · Leptin · Autism · Regression

Introduction

Autism spectrum disorders (ASD) are complex neurodevelopmental disorders distinguished by impaired

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social interaction, deficits in verbal and non-verbal communication, and restricted repetitive and stereotyped patterns of behavior and interests (Filipek et al., 2000; Lord et al., 2000). ASD is phenotypically heterogeneous and may include several diseases with distinct etiologies and pathogenic mechanisms. Children with ASD can be clinically differentiated as having early onset or regressive autism. Early onset autism refers to children with early delays in the development of language and/or social skills, while regressive autism refers to those who develop some language and/or social skills that are subsequently lost usually between 18–24 months of age (Lainhart et al., 2002).

There is mounting evidence of an immunological dysfunction in children with autism. Studies have demonstrated an increased frequency of autoantibodies to brain/central nervous system proteins (Plioplys, Greaves, & Yoshida, 1989; Singh & Rivas, 2004; Vojdani et al., 2002), increased proinflammatory cytokine levels and decreased anti-inflammatory cytokine levels (Ashwood, Anthony, Torrente, & Wakefield, 2004; Jyonouchi, Sun, & Itokazu, 2002) and skewed TH2 cytokine profiles (Gupta, Aggarwal, Rashanravan, & Lee, 1998). An altered immune response may impact other biological systems including the neuroendocrine and nervous systems and vice versa. One of the most interesting of the neuroendocrine (Chandra, 1980) mediators recently shown to have an effect on the immune system is leptin.

Leptin shares structural and functional similarities with several cytokines, many of which are involved in neurodevelopment, including IL-6 and IL-12 (Zhang et al., 1994). While adipocytes are the major source of leptin, production by lymphocytes has also been demonstrated (Sanna et al., 2003). Several studies suggest a role for leptin in immune modulation. Mice lacking leptin have defective cell-mediated and humoral immunity (Chandra, 1980). Similarly, humans with congenital leptin deficiency are more susceptible to fatal infections during childhood (Ozata, Ozdemir, & Licinio, 1999). In animals with autoimmune diseases, leptin deficiency has been shown to shift the immune response from TH1 to TH2, leading to a protective effect against inflammation (Busso et al., 2002). Importantly, inflammatory cells themselves secrete leptin, which may further foster the inflammatory process (Fantuzzi et al., 2005; Sanna et al., 2003; Xu et al., 2003). In the following study, we compared plasma leptin levels in a population of well-defined children with autism and age-matched controls, with and without developmental disabilities.

Materials and Methods

Subjects

This case-control study examined 169 children, ages 2–15, enrolled both through the M.I.N.D. (Medical Investigations of Neurodevelopmental Disorders) Institute clinic (n = 100), and the Center for Children's Environmental Health (CCEH) (n = 69) as part of the ongoing CHARGE (Childhood Autism Risk from Genetics and Environment) study at UC Davis (Hertz-Picciotto et al., 2006). The M.I.N.D. clinic sample population consisted of children diagnosed with autism and their typically developing siblings, and a control population of age-matched typically developing children as determined by medical record abstraction. The CHARGE study population was sampled from three strata: children with autism, children selected from the general population without autism or other developmental disabilities, and children with developmental disabilities without autism. Samples from siblings of the CHARGE autism cases, who did not themselves have autism, were also included in this study. Statistical differences observed with the complete data set were unaffected when performed on the M.I.N.D. samples alone. Therefore, all the data presented herein reflects the combination of both sample populations.

To confirm and further detail the initial diagnosis, all children were assessed at the UC Davis M.I.N.D. Institute. Autism was confirmed for all cases using the Autism Diagnostic Interview-Revised [ADI-R; (Lord et al., 1997)] and the Autism Diagnostic Observation Schedule, modules 1, 2 or 3 [ADOS; (DiLavore, Lord, & Rutter, 1995; Joseph, Tager-Flusberg, & Lord, 2002; Lord, Leventhal, & Cook, 2001; Owley et al., 2001)]. The ADI-R provides a standardized, semi-structured interview and a diagnostic algorithm for the DSM-IV (American Psychiatric Association 1994) and the ICD-10 definitions of autism (World Health Organization (WHO), 1992) (Steinhausen & Erdin, 1992). The ADOS is a semi-structured, standardized assessment in which the researcher observes the social interaction, communication, play and imaginative use of materials for children suspected of having ASD. Final autism case diagnosis was defined as meeting criteria on the communication, social, and repetitive behaviors domains of the ADI-R and scoring at or above the cut off for autistic disorder on the ADOS module 1 or 2. The Social Communication Questionnaire was used to screen for behavioral and developmental characteristics of ASD among the subjects with developmental disabilities and among the typically developing controls;

children who scored above the screening cut-off were fully assessed using the ADI-R and ADOS. Only those exceeding the cut-off were included in the study.

The sample population included 70 children diagnosed with autism, 50 age-matched typically developing controls, 26 siblings of children with autism, and 23 children diagnosed with developmental disabilities [descriptive statistics presented in Table 1]. Due to the relationship between plasma leptin levels and Body mass index (BMI), calculations for BMI were made for all groups. As this is a pediatric study, a more relevant measure for BMI in children, also referred to as BMI-for-age, was determined as a measure of growth specific for gender and age (Hammer, Kraemer, Wilson, Ritter, & Dornbusch, 1991; Pietrobelli et al., 1998). The BMI-for-age is calculated based on gender-specific growth charts that can then be used to calculate the BMI Z-score, or the degree of standard deviation from the standardized growth chart for children and teenagers 2–20 years of age. BMI-for-age calculations were made for all groups using standard reference charts from the Centers for Disease Control (CDC) that are based on a representative US population measured in 2005 (http://www.cdc.gov/nccdphp/dnpa/bmi/bmi-for-age.htm) (Table 1).

The children with autism were further subdivided into children who initially developed normally, reaching typical developmental milestones before regressing and losing language and social skills, and those who had early impairments in the development of language and social skills. A classification of regression was based on clinical characteristics using both parental reporting and answers to questions regarding language loss (Q11) and social skills (Q25) of the ADI-R. Our autism study population could be classified into 37 subjects with early onset autism (classical) and 33 subjects with delayed-onset autism (regression). The study protocol followed the ethical guidelines of the most recent Declaration of Helsinki (Edinburgh, 2000), and was approved by the Institutional Review Boards of the UC Davis School of Medicine and the State of California, and all subjects enrolled in the study had written informed consent provided by their parents and assented to participate if developmentally able.

Determination of Plasma Leptin Concentrations

Peripheral blood samples were drawn in ACD solution Vacutainers (BD Biosciences, San Jose, CA), samples were centrifuged for 10 min at 2300 rpm at room temperature and the plasma harvested and frozen at –80°C. Plasma leptin concentrations were measured using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Linco Research, St. Charles, MI). Samples were run in duplicate and in concordance to the Sensitive Assay instructions of the kit protocol. Plasma aliquots had undergone no more than 1 freeze/thaw cycle.

Statistical Analysis

Statistical analyses were performed using R statistical analysis software (http://www.r-project.org/). A Shapiro-Wilk test was used to assess the normality of both the leptin data set, and log-transformed leptin levels. In either case, the test indicated non-normality of the data ($p < .005$). The Kruskal–Wallis rank sum test was used to compare leptin levels between groups. Data are expressed as median values (interquartile ranges). A Mann–Whitney non-parametric *U*-test (with a Holm step down procedure to correct for multiple comparisons) was used in post-hoc analyses to compare leptin levels between groups and adjusted p values <.05 were considered statistically significant. Because of the lack of normality of leptin and log-transformed leptin

Table 1 Descriptive statistics of the study population

	Autism			Typically developing controls (n = 50)	Siblings (n = 26)	Developmental disabilities (n = 23)
	Total (n = 70)	Early onset (n = 37)	Regression (n = 33)			
AGE:						
Median (Interquartile range)	4.25 (2.4–15.5)	3.83 (2.4–15.5)	4.58 (2.5–14.6)	4.33 (2.2–14.7)	5.1 (1.8–12.5)	3.38 (2.0–5.0)
Male%	87	86	88	76	77	87
BMI:						
Median (Interquartile range)	16.79 (16.01–17.94)	17.28 (16.41–17.5)	16.48 (15.91–18.66)	16.68 (16.11–17.18)	18.02 (16.73–19.28)	17.02 (16.04–17.73)
BMI-for-age Z-scores (Range)	0.75 (–1.5 to >2)	0.5 (–1.5 to >2)	1.0 (–1 to >2)	0.5 (–2 to 2)	0.5 (–2 to 2)	1.0 (0 to >2)

values, a nonparametric ANCOVA analysis (sm.ancova subroutine in the R-statistical analysis software)- which allows a set of nonparametric regression curves to be compared- was used to determine whether age or BMI differences between the study groups was a confounding factor that could account for group differences in the leptin levels.

Results

Leptin levels were significantly higher in children with autism compared with typically developing non-ASD controls (median = 2.11, interquartile range (0.87–3.4) ng/ml versus 0.96 (0.49–1.66) ng/ml, $p < .006$). Individual results are represented in Fig. 1. Similarly, children with autism had higher plasma leptin values compared with siblings (1.19 (.78–2.08) ng/ml, $p < .026$) and children with developmental delay (0.88 (.43–2.07) ng/ml, $p < .012$). In contrast, no differences in leptin levels were seen between siblings of subjects with ASD, the typically developing controls and children with developmental delay ($p > .05$).

Strikingly, when the study subjects were categorized based on clinical features a significantly higher level of plasma leptin was observed in children with early onset autism compared with those with clinical regression (2.62 (1.12–4.38) versus 1.38 (0.61–2.69) ng/ml, $p < .042$) (Fig. 2). Moreover, children with early onset autism had significantly higher leptin levels compared with typically developing age-matched controls ($p < .0015$), and children with developmental delay ($p < .004$).

In contrast, autism children with regression were not different when compared with the typically developing children or with those who were developmentally delayed. There were no statistical differences in BMI or Z-scores between autism cases or controls. Furthermore,

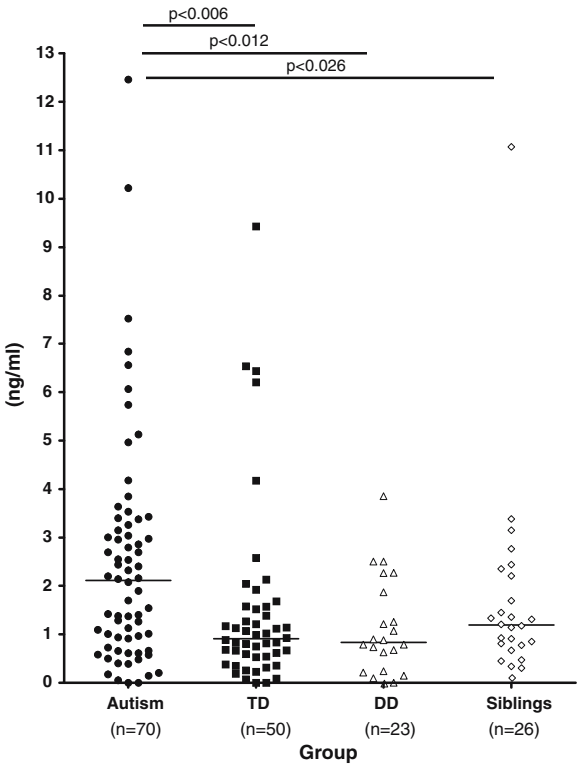

Fig. 1 Leptin levels (ng/ml) in children with autism and age-matched controls. A significant difference in leptin levels was noted between the autism group and typically developing non-ASD controls (TD) ($p < .006$), controls with developmental disabilities (DD) ($p < .012$), and siblings of children with autism ($p < .026$). Outliers for autism ($n = 2$) (minimum–maximum ranges 0–33.91), TD ($n = 2$) (range 0–31.02), and DD ($n = 1$) (range 0–24.26) were excluded from the figures but included for the statistical analysis

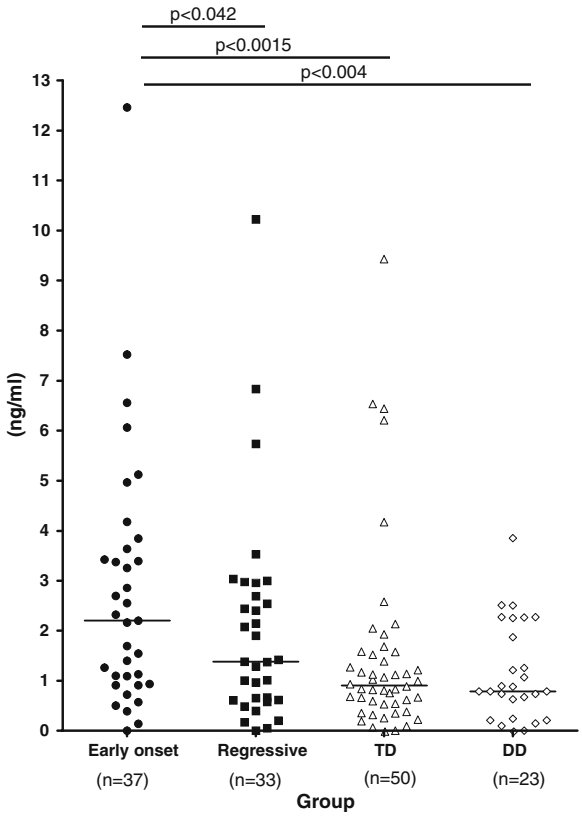

Fig. 2 Leptin levels (ng/ml) in children with distinct autism phenotypes. Strikingly, a significant difference in the plasma leptin levels was noted between the subjects with early onset autism compared with those with regressive autism ($p < .042$). In addition, plasma leptin levels were significantly elevated in early onset autism (range 0–33.91) when compared with the typically developing controls (TD) ($p < .0015$), and with the developmental disabilities (DD) controls ($p < .0036$). Plasma leptin levels for those subjects with regressive autism (range 0–10.22) were similar to all control groups

a nonparametric ANCOVA analysis showed that neither age, IQ, nor differences in BMI or Z-scores was a confounding factor that could account for differences in the leptin levels between the groups. In accordance with previous studies, we observed that leptin levels were sexually dimorphic, with higher levels in females than males (Garcia-Mayor et al., 1997). When only the male subjects in each group were compared, the same patterns of statistical significance as detailed above were observed (data not shown). There were too few female subjects to make meaningful comparisons between groups. No differences were noted in autism cases that were on medications compared with untreated autism cases ($p > .1$).

Discussion

Autism spectrum disorders represent a group of phenotypically diverse neurodevelopmental syndromes that manifest early in development. In search of biological correlates for ASD, we investigated the plasma concentration of leptin in children with autism compared with age-matched typically developing controls, siblings, and children with developmental delay in cognitive or adaptive function but not autism. The current study describes a significant increase in plasma leptin levels in children with autism when compared with each of these groups. Furthermore, the elevation in leptin concentration was primarily attributed to subjects with a clinical phenotype of early onset autism while levels in the autism population with regression did not markedly differ from the control groups.

Originally discovered as a regulator of body-weight, leptin is secreted from adipose tissue into the blood where it mediates feeding behavior and thermogenesis (Banks, 2004; Banks & Lebel, 2002; Kastin, Pan, Maness, & Banks, 1999). Leptin has recently been shown to have wide ranging immunological effects (Caldefie-Chezet, Poulin, & Vasson, 2003; Howard et al., 1999; Mancuso et al., 2002) and can modulate TH1 autoimmunity through receptors on CD4+ T lymphocytes (Steinman, Conlon, Maki, & Foster, 2003). Leptin may be considered a pro-inflammatory cytokine that perpetuates inflammation through the differentiation of TH1 cells and enhanced cytokine production (Matarese, Moschos,& Mantzoros, 2005; Sanchez-Margalet et al., 2003).

The differential leptin levels reported herein are not associated with differences in BMI-for-age Z-scores or age, and therefore may reflect altered immune function. Furthermore, the trend toward higher leptin levels in the early onset phenotype may signify a distinct neuropathologic mechanism resulting in different clinical behavioral outcomes. The basis for dichotomizing the subjects into early onset and regressive phenotypes in the current study stems from the recent data that suggest differing etiologies between these two behavioral phenotypes (Richler et al., 2006).

There is precedent for the involvement of leptin in neuropathology. First, leptin can cross the blood brain barrier through active transport (Banks, 2001). Furthermore, leptin deficient mice are resistant to induced experimental autoimmune encephalomyelitis (EAE), a model of multiple sclerosis, showing reduced T cell responses to myelin antigens and increased IL-4 production. However, these mice become susceptible to EAE after administering leptin, a finding that is consistent with its TH1-promoting activities (Matarese et al., 2001) and which further demonstrates its potential role in neuropathology. Moreover, immune cells infiltrating central nervous system lesions in these mice stain positive for leptin, suggesting a local production of leptin during acute EAE (Matarese, Moschos, & Mantzoros, 2005). Finally, Vargas, Nascimbene, Krishnan, Zimmerman, & Pardo (2005) recently found increased leptin levels at the sites of inflammation in post-mortem brain tissue from individuals with autism (Vargas et al., 2005).

In conclusion, we found that plasma leptin levels were elevated in a population of children with autism compared to typically developing controls. Notably, this elevation was exaggerated in children with early onset autism rather than those with clinical regression. It is currently unknown if altered leptin levels are active determinants in the development of neuropathology in autism, or accompanying phenomena secondary to the onset of the disease. Nonetheless, these findings provide a framework for further longitudinal studies to investigate changes in leptin levels over the lifetime of the disorder. Finally, a key question remains regarding the concentration of leptin at birth and/or in early development in the autism subjects with early onset disease. Future studies evaluating leptin levels during critical periods of neurodevelopment would be essential towards addressing the potential role of leptin as a biological component in autism.

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